

# Etude sur la Transmission de la Maladie de Cap Saint Paul du Cocotier au Ghana

## *Study on the Transmission of Coconut Cape Saint Paul Wilt Disease (CSPWD) in Ghana*

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### Résumé :

Les recherches sur le vecteur de la maladie de Cap Saint Paul du cocotier au Ghana ont débuté à partir de 1990 (90-97; 2002-04) et n'ont pas donné de résultats probants. A partir de juillet 2005, de nouvelles normes d'essais ont été appliquées : ombrière, collectes et lâchers journaliers d'insectes aux heures les moins chaudes, cages et plants de cocotier de différentes tailles. On a introduit en cage 73000 *M. adiopodoumeensis* pendant 28 mois (520 adultes/plant/mois). Des contrôles en PCR sur les 5 cocotiers de cette cage *Myndus* et sur 935 adultes ont toujours été négatifs. Les essais de transmission avec *M. adiopodoumeensis* apparemment non vecteur de la maladie, ont été donc arrêtés. Le phytoplasme du CSPWD a été identifié par PCR dans un cocotier ayant reçu 4380 *Diosmombus* (4 espèces de Derbidae) 4 mois après le début des lâchers. Ce cocotier n'a jamais présenté de symptôme de maladie 28 mois plus tard et toutes les PCR successives ont été négatives. Les Auchenorrhynches collectés par fauchage sur les adventices dans et autour de la parcelle pendant le jour, ont été également testés sans succès. L'hypothèse d'une transmission par le sol est également prise en compte à cause de la présence de cochenilles et de nématodes.

**Mots clés :** Jaunissement mortel – vecteur - Homoptères – Auchenorrhynches – cochenilles – cocotier – Ghana.

### Abstract:

Research on the Cape Saint Paul Wilt Disease (CSPWD) vector in Ghana began as from 1990 (90-97; 2002-04) and did not give convincing results. From July 2005, new tests standards were applied: shading, daily collections and releasing of insects at the less hot hours and use of various sizes of cages and test plants. More than 70000 *M. adiopodoumeensis* were introduced in cage for 28 months (520 adults/seedling/month). Controls in PCR on the 5 coconut of this *Myndus* cage and on 935 adults were always negative. The tests of transmission with *M. adiopodoumeensis* apparently not a vector of the disease, were thus stopped. The Phytoplasma of the CSPWD was identified by PCR in a coconut having received 4380 *Diosmombus* (4 species of Derbidae) 4 months after the beginning of the test. This coconut never presented symptom of the disease 28 months later and all the successive PCR were negative. Auchenorrhyncha collected by sweeping on the adventitious plants in and around the plot during the day, were also tested without success. The hypothesis of a ground transmission was also taken into account because of the presence of scale insects and nematodes.

**Key words:** Lethal yellowing - Vector - Homoptera - Auchenorrhyncha – Scale insects - Coconut - Ghana.

### Resumen: Investigacion sobre el insecto vetor del amarillamiento letal del cocotero en Ghana

Las investigaciones sobre el vector del Cape Saint Paul Wilt Disease (CSPWD) en el Ghana empezaron desde el año 1990 (1990-97; 2002-04) sin dar ningún resultado convincente.

A partir de julio 2005, nuevos dispositivos fueron aplicados: se colocaron sombras, se capturaron y se soltaron los insectos en las horas menos calientes del día, y se instalaron jaulas y palmas de cocotero de diferentes tamaños. Se introdujeron en jaula 70000 *Myndus adiopodoumeensis* en un periodo de 28 meses (520 adultos /planta /mes). Los controles PCR en los 5 cocoteros de esta jaula "Myndus" y en 935 adultos fueron todos negativos. Los ensayos de transmisión con *M. Adiopodoumeensis*, manifiestamente no vector de la enfermedad, fueron entonces suspendidos. El fitoplasma del CSPWD fue identificado por PCR en un cocotero que había recibido 4380 *Diosmombus* (4 especies, Derbidae) 4 meses después de soltar los primeros insectos. Este cocotero nunca mostró síntoma de enfermedad hasta 28 meses mas

tarde y todas las PCR siguientes fueron negativas. Los Auchenorrhynchas cogidos de día, pasando red de golpeo en las adventicias de la parcela y sus alrededores, fueron también testados sin éxito. La posibilidad de una transmisión por el suelo fue también tomada en cuenta en razón de la presencia de cochinillas y de nematodos.

**Palabras claves:** Amarillamiento letal – vector – Homopteros – Auchenorrhyncha – cochinillas – cocotero – Ghana.

## Introduction

Phytoplasmas are known to be associated with coconut Lethal Yellowing. It is also known that all phytoplasma diseases are transmitted by leafhoppers (Membracidae, Cicadellidae), planthoppers (Delphacidae, Cixiidae, Derbidae, etc.) or by Psyllidae (Weintraub, 2006). These Homoptera Auchenorrhyncha also play a role in transmitting viral diseases of palms (Julia, 1982; Nault et al 1989).

To date, only the Cixiidae *Myndus crudus* has been experimentally identified as an effective vector of LY (Lethal Yellowing), and only in Florida (Howard, 1983). Thus, it is only in Florida that it has been possible to detect through an electronic microscope the existence of the LY phytoplasma in the body of *M. crudus*. At that time, there was no check by PCR. Cloning and sequencing were not in practice. So, it is difficult to say that the Phytoplasma observed was really that of coconut Lethal Yellowing in Florida.

However, it has so far been impossible to confirm the role of *Myndus* as a vector in Jamaica or Mexico.

In Tanzania, the Derbidae *Dioscrombus mkurungai* Wilson and *Meenoplus* sp (*Meenoplidae*) are suspected by the sole fact that LY phytoplasma has been detected by the PCR technique (Mpunami et al. 2000).

Identification of vector insects by experimentally reproducing disease symptoms under controlled conditions is essential (Philippe et al. 2007). It will enable optimum application of all the research to be undertaken for a better understanding of the epidemiology and for designing control methods.

We describe here the work being conducted in Ghana to search for the Cape Saint Paul Wilt (CSPWD) vector.

## 1. History of disease transmission studies in Ghana

The first disease transmission studies were undertaken with World Bank funding from 1990 to 1992. A European Union project (STD 3) then took over from 1993 to 1997.

Starting in 1990, 18,000 to 20,000 *Myndus adiopodoumeensis* Synave (Cixiidae), and around 20,000 Derbidae and other Homoptera were tested in large cages (6.7 x 6.7 x 7.5m) with one 6 to 7-year-old WAT per cage. Some enigmatic results were obtained, which were not backed up by PCR tests at the time (Dery et al. 1995, 96).

Starting in January 1996, attempts were made in medium sized cages to transmit the disease with the same species after spending 3 days inside a large sleeve on a diseased coconut palm. The aim was to check for the existence of the phytoplasma in the insects and in the nursery plants using the PCR analysis method. A PCR analysis laboratory was set up by DFID (ex-NRI) at the University of Legon (Accra). The nursery plants did not display any yellowing symptoms and the PCR analyses were negative.

From the end of 2002 onwards, an AGSSIP (Agricultural Services Sub-Sectors Investments Programme) budget made it possible to relaunch two series of disease transmission trials. The insects also underwent an acquisition phase. No positive PCR case was obtained. In the second series, a plant onto which all the Derbidae species had been released gave a positive PCR analysis. DNA sequencing revealed that it was not that of the phytoplasma responsible for CSPWD (Cape Saint Paul Wilt).

## 2. New transmission studies under project FSP 2004-34

The CSPWD transmission trials began in July 2005 with new experimental conditions:

- Construction of a shade over each cage
- Insects were collected in the morning from 6 am to 8 am and in the evening from 4 pm to 5.30 pm.
- Those insects were released immediately into the respective cages after each of the 2 daily collections.

## 2.1. Material and method

### 2.1.1. Medium sized cages

An initial set of 5 shaded cages (2.2m x 2.2m x 2.2m) housed *Myndus* and Derbidae; Homoptera collected with sweep nets from the plant cover under the coconut palms and around the coconut plot; a control with shade; a control without shade – six 12 to 18-month-old nursery plants (3 Malayan Red Dwarf palms=MRD and 3 Malayan Yellow Dwarf palms=MYD) per cage; each plant was fitted with a sleeve.

A medium sized cage was reserved for the small and large sucking insects collected at the two periods of the day using a sweep net in the herbaceous cover inside and around the plot.

Those insects belonged to 2 major groups of Homoptera: leafhoppers (Cicadellidae and Cercopidae) and planthoppers (Delphacidae, Achilidae, Meenoplidae); along with a few Heteroptera (Pentatomidae).

- 1st series: 6 nursery plants (3 MRD and 3 MYD) aged 12 to 18 months per cage; each plant was fitted with a sleeve. From July to December 2005, it proved possible to release a total of 9,910 insects, i.e. an average of around 275 insects per plant per month.

- 2nd series: Two plants were kept (1 MYD and 1 MRD) from the previous six. One plant received only small leafhoppers, the other only large leafhoppers + Pentatomidae + Flatidae + Cercopidae (Table 2).

- 3rd series: Two new plants were introduced (MYDxVTT=Vanuatu Tall) (Table 2).

### 2.1.2. Large cages

#### - *Myndus adiopodoumeensis*<sup>2</sup>

A large cage (3 x 3 x 3.5m) was constructed to house 5 hybrid coconut seedlings (MYD x VTT) not covered with a sleeve.

#### - *Derbidae*<sup>1</sup>

Three large cages (4m x 4m x 4m) housed respectively: 4 species of *Diostrombus*: *D. mayumbensis*, *D. nitidus*, *D. luteus*, *D. dilatatus*; *M. stellulata* + *Patara armata*; 1 control cage. These insects were released onto three plants (MYDxVTT) over 2 years old, placed in each cage without sleeve.

Nota bene: The cages have a red wooden or metal (angle iron) frame and they are covered with a polyester sleeve (Fyltis) with 600 or 800 meshes.

## 2.2. Results

### 2.2.1. With medium sized cages

#### - *Myndus adiopodoumeensis*

This test was halted after 4 months and the plants were kept under a veranda that was sunny but protected from any visiting Homoptera. None of the coconut plants receiving 1,400 *M. adiopodoumeensis* adults for 4 months displayed any disease symptoms 27 months after the trial was halted.

#### - *Derbidae*

Three groups of 2 plants each fitted with a sleeve were placed in the medium sized Derbidae cage: 1st group with *Metaphenice stellulata* (Boheman), a species in which a phytoplasma different from that of CSPWD has been found; 2nd group with *Patara armata* Van Stalle; 3rd group with 4 species of *Diostrombus* in a mixture: *D. mayumbensis* Synave, *D. dilatatus* (Westwood), *D. luteus* (Muir), *D. nitidus* Muir.

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<sup>2</sup> Homoptera Auchenorrhyncha determinations were carried out by Jacques Bonfils, a retired entomologist, Pentatomidae and Cercopidae by the CIRAD faunistics laboratory at Baillarguet (Henri-Pierre Aberlenc & Jean-Michel Maldès)

**Table 1:** Tests of Homoptera Auchenorrhyncha for LY transmission on coconut palms aged 12 to 18 months

Species	July to Nov. 2005 (Average insects/plant)	Dec. 2005 to Oct. 2006 (Total insects/1 single plant)
4 species of <i>Diosmombus</i>	4,380	24,050
<i>Metaphenice stellulata</i>	1,320	10,030
<i>Patara armata</i>	1,800	5,500

One (1) plant that received an average of 4,380 *Diosmombus* (Table 1), tested positive in **direct** PCR. In addition, the **DNA was identified after cloning and sequencing as being that of the LY phytoplasma**. Unfortunately, that plant did not display disease symptoms. Subsequent PCR analyses (2 and 4 months later) did not reveal the presence of the phytoplasma.

That may have been due to the fact that:

- the plants were too young and the sap did not offer good conditions for phytoplasma development and
- the plants under shade were not traumatized and were able to more effectively resist any phytoplasma aggression.

This plant was also kept under the sunny veranda protected from any visiting Homoptera. It did not express any disease symptoms 27 months after insect releases were halted. It should be noted that these species remained on the underside of leaflets throughout the day.

#### **- Other Homoptera collected in sweep nets**

This test was organized to try and see if the vector might be sheltering in the plant cover of the coconut plot or the surrounding area during the day. So far, two-monthly PCR analyses have yet to reveal the existence of phytoplasma in coconut plants subjected to the pressure of more than 40 species of Cicadellidae (small and large), Pentatomidae, Cercopidae and Flatidae.

**Table 2:** Tests of Homoptera collected in sweep nets for LY transmission on coconut palms aged 12 to 18 months

Species	Jan 2006 to Oct. 2006 (Total insects/3 plants/10 months)	Average insects /month/plant
Small Cicadellidae	4,801	160
Large Cicadellidae + Cercopidae + Pentatomidae + Flatidae	12,822	427
<b>TOTAL Jan. to Oct. 2006</b>	<b>17,623</b>	<b>587</b>
Species	Nov. 2006 to March 2008 (Total insects/2 plants/17 months)*	Average insects /month/plant
Small Cicadellidae	9,498	279
Large Cicadellidae	22,343	657
Pentatomidae	421	12
Cercopidae	3,435	101
Flatidae	275	8
<b>TOTAL Nov. 06 to March 08</b>	<b>35,972</b>	<b>1,058</b>

\* The plants of the second series from Nov. 2006 to March 2008 were different from those in the first series from Jan. to Oct. 2006.

#### **- Other so-called "rare" Homoptera collected from fronds**

Since November 2006, other Homoptera found at a very low frequency on fronds have been released into a medium sized cage; these are in decreasing order of abundance: *Proutista fritillaris* (Boheman) (Derbidae), *Nibia nervosa* (Motschulsky) (Meenoplidae), *Diosmombus annetti* (Muir), *Diosmombus nitidus* Muir (Derbidae) (Table 3), and others in very small numbers but not counted such as the Derbidae, *Zorabana maculata* Van Stalle, *Pamendaga nealei* (Distant), *Diosmombus* (*Lyddasmombus*)

*lineaticeps* (Muir), the Meenoplidae *Anigrus lugens* (Muir) and *Meenoplus stramineus* (Stal), and a species of Lophopidae, *Elasmoscelis trimaculata* Walker. So far, none of the coconut seedlings that have received the pressure of some tens to some thousand insects has yet expressed the symptom of the disease.

**Table 3:** Tests of so-called "rare" Homoptera Auchenorrhyncha for LY transmission on coconut palms aged 12 to 18 months

Species (Families)	Nov. 2006 to March 2008 (Total insects/2 plants/17 months)	Average insects/month/plant
<i>Proutista fritillaris</i> (Derbidae)	3,421	101
<i>Nibia nervosa</i> (Meenoplidae)	1,286	38
<i>Diostrombus annetti</i> (Derbidae)	201	6
<i>Diostrombus nitidus</i> (Derbidae)	728 (since Nov. 07)	21
<i>Lophopidae</i> sp	418	12
Other species	50	1

### 2.2.2. Large cages

#### - *Myndus adiopodoumeensis*

The main problem encountered is the high mortality of *Myndus* inside the cage: only between 0 and 5% of insects introduced were alive the next morning. *M. adiopodoumeensis* were excessively attracted to light. They therefore flew to the Fyltis netting and only rarely visited the coconut fronds. On the Fyltis netting they soon died from exposure to the sun; they were caught in spider webs in the upper corners of the cages or they were attacked by ants. *Myndus* flights to the Fyltis netting were greatly reduced by installing mosquito netting over the plants. The adults thus remained longer on the fronds. Consequently, 46% of released insects were still alive the next morning. However, despite this substantial improvement of their conditions in captivity, the adults do not survive more than 4 days (Figure 1). The adults, if they were under the leaflets, were not moving much, but it is difficult to determine exactly if they were in position to stick in the leaflet and withdraw the sap or not.

So far, the 5 coconut seedlings have not yet expressed the symptom of the disease after 28 months despite the number of *M. adiopodoumeensis* released per plant and per month (Table 4). It is therefore unlikely that this species is the vector of the CSPWD phytoplasma.

**Table 4:** Tests of *M. adiopodoumeensis* for LY transmission on coconut palms around 2 years old

Species	Dec. 2005 to March 2008 (Total insects/5 plants/28 months)	Average insects/plant/month
<i>M. adiopodoumeensis</i>	72,943	521

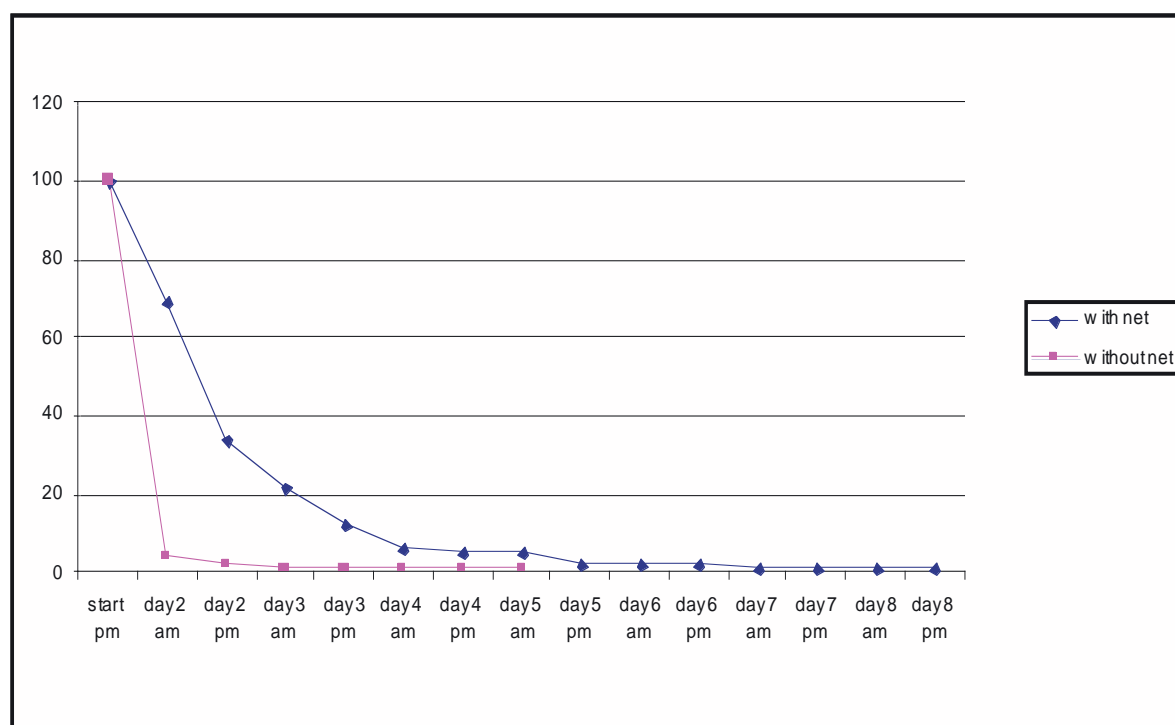
#### - *Derbidae*

The table 5 indicates the Derbidae spp tested and their numbers released in large cages. Derbidae mostly remained on the coconut palms. They were not much attracted by the Fyltis netting and sunlight. Nevertheless, mortality was not insubstantial in most of the species tested. The most resistant species to heat was *D. dilatatus*.

The height of the cages (4 m) made it difficult to construct a bamboo or thatch shade which would quickly be destroyed by a strong gust of wind (occurring approximately annually). Percale fabric (flour bags) was tested and gave sufficient shade to reduce the temperature by 2°C. Apparently, that drop in temperature inside the cage did not lead to a significant drop in insect mortality. This suggests that the confinement aspect (absence of air circulation) might play a definite role in insect survival in the cage. Remember that outside the maximum temperature was around 35 to 36°C but air circulation makes the heat more bearable.

So far, the 2 batches of 3 coconut seedlings more than 2 years old have not yet expressed the symptom of the disease after 17 months (Table 5).

**Figure 1:** Survival of *M. adiopodoumeensis* with or without mosquito net



**Table 5:** Tests of Derbidae for LY transmission on coconut palms around 2 years old

Species	Nov. 2006 to March 2008 (Total insects/3 plants/17 months)	Average insects/month/plant
<i>D. mayumbensis</i>	21,348	418
<i>D. nitida</i>	661	13
<i>D. luteus</i>	8,673	170
<i>D. dilattatus</i>	8,395	164
<i>M. stellulata</i>	24,496	480
<i>P. armata</i>	11,811	231

### 3. Other observations

#### 3.1. Nematodes<sup>3</sup>

A sample of soil + pieces of roots, taken from around two diseased coconut palms, was analysed in the Institute of Research for Development (IRD) at Montpellier (France). The roots were virtually uninfested, as the *Pratylenchus* population levels were very low. On the other hand, the *Helicotylenchus*, especially *Heterodera* (cyst nematode) and *Meloidogyne* (root-knot nematodes) were pretty high. No cysts or root-knots were found on the roots.

The nematodes, vectors of viruses, practised only mechanical transmissions through their stylet, which makes it very unlikely the transmission of Phytoplasma. Nevertheless, this possible soil effect is being taken into account in our transmission trials with 5 cages (2.5 x 2.5 x 2.5 m) in which the coconut were planted directly in the soil without any insects releasing in the cages.

<sup>3</sup> Determination by Thierry Mateille, IRD Nematologist - UMR 1062 INRA/IRD/SUPAGRO/CIRAD/ CBGP - Campus de Baillarguet - France



### 3.2. Soil scale insects<sup>4</sup>

Scale insect searches have been resumed in the soil specifically around diseased coconut palms. It has been possible to detect five species of Pseudococcidae: *Geococcus coffeae* Green, *Planococcus minor* (Maskell), *Rhizoecus* sp, *Ferrisia virgata* (Cockerell), *Paracoccus* sp and one species of Aphididae: *Geioica* sp. So far, no Margarodidae have been found (ground pearls).

Control experiments with nematodes and scale insects prove very difficult. In fact they are conducted blind, i.e. every month the soil in the large tubs containing the tested coconut palms is changed in two cages. Soil is taken from around diseased coconut palms; it also contains pieces of diseased coconut palm roots.

### 3.3. Insects in frond axils

Searches with a brush in frond axils have revealed some larvae of a sucking species. The eggs were placed in a hatchery and gave a Derbidae known as *Metaphenice stellulata*. A Derbidae species is known thereby to complete its entire cycle on coconut.

Another species of white larvae (probably Cicadellidae) was spotted once in the plant debris in frond axils, without obtaining any adults, which makes impossible the determination of the species.

### 3.4. Insects on inflorescences

Thrips are known to exist on coconut inflorescences. Observations of a series of inflorescences dissected on flowering revealed the effective existence of a black Thrips species.

## 4. Discussion and prospects

The search for the coconut LY vector in Ghana was launched following observation of the existence of *M. adiopodoumeensis* species in the first focus at Ayensudu which started in 1984. In fact, that species of Auchenorrhyncha which is well-known as an intra-phloemic sucking species is already responsible for the transmission of a phytoplasma in Florida, with the species *M. crudus*, and of a viroid in Vanuatu with the species *M. taffini*. At the same time like *M. adiopodoumeensis*, all the other diurnal species found on the underside of leaflets have been tested. They belong to the families of the Derbidae: some are abundant like certain species of *Diostrombus* and others less frequent like *Proutista*. The Meenoplidae are not very frequent, likewise the Lophopidae.

More than 40 species of Cicadellidae, Pentatomidae, Cercopidae and Aphrophoridae harvested with sweeping net, twice daily, among the weeds or cover crops inside and around the experimental plot, have also been tested in a medium cage.

Investigations were then extended to insects (scale insects) and other organisms (nematodes) in the soil. This soil effect needs to be taken into account without being able to set up precise trials as it is difficult to collect a large number of scale insects which are very delicate to handle. Tests with nematodes also present the same problems in practice (collection, counting of individuals, releases, mortality monitoring, etc.).

The diurnal investigations therefore seem complete and no disease symptoms have been expressed in cages after two and a half years. Nocturnal observations have therefore begun, collecting insects directly from leaflets. Dozens of species of Cicadellidae not found in the daytime with sweeping net have been collected in that way.

Two groups seem to be of interest: Cicadellidae of the sub-family Deltocephalinae which contains numerous species that are potential disease vectors, along with the family of the Delphacidae.

The next tests in cages will be conducted with species from these two groups of insects.

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<sup>4</sup> Determination by Jean-François Germain from the National Plant Protection Laboratory – Entomology Unit - Montpellier - France

## 5. Conclusions

Results of PCR analyses showed that a species of Derbidae might potentially be the disease vector. First of all, a coconut plant around 1 year old was infested by a group of 4 *Diostrombus* (Derbidae). However, disease symptoms had yet to occur on the plant 18 months after the phytoplasma was detected. It may be that the age of the plant was not favourable for pathogen development. It tested negative on subsequent occasions.

After 27 months of trials meticulously conducted in Ghana with the species *M. adiopodoumeensis*, more than 70,000 adults have been released on 5 seedlings in insect-proof cage. So, in the absence of any positive PCR analysis responses in coconut palms in cage, it turns out that this species seemed not be the disease vector in our experimental conditions.

In addition, none of the coconut plants exposed in cages to whatever species of Homoptera Auchenorrhyncha has expressed any lethal yellowing symptoms to date.

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